

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
18 March 2004 (18.03.2004)

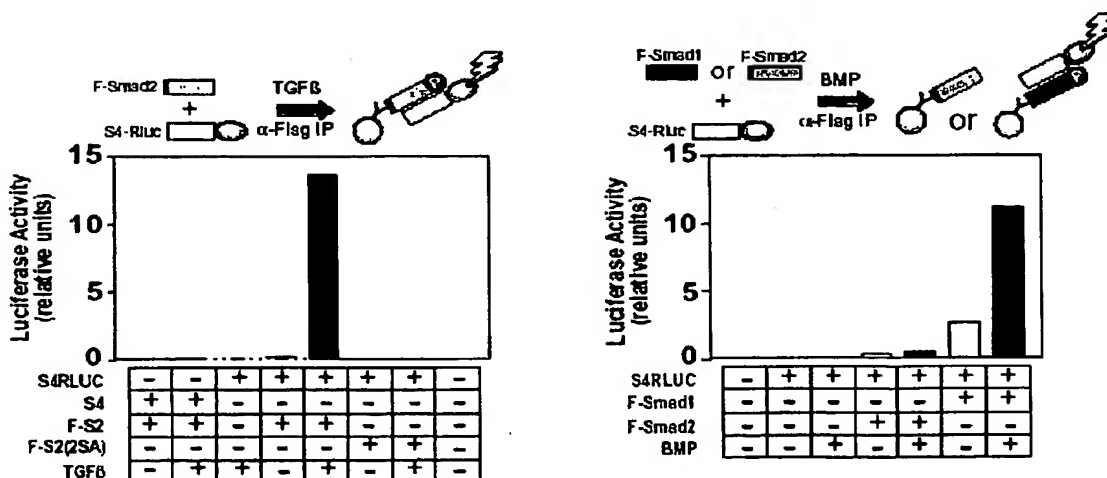
PCT

(10) International Publication Number
WO 2004/023146 A3

- (51) International Patent Classification⁷: **G01N 33/68**, C12Q 1/66
- (21) International Application Number: **PCT/CA2003/001354**
- (22) International Filing Date: **5 September 2003 (05.09.2003)**
- (25) Filing Language: **English**
- (26) Publication Language: **English**
- (30) Priority Data: **60/408,922** **6 September 2002 (06.09.2002)** **US**
- (71) Applicant (for all designated States except US): **MOUNT SINAI HOSPITAL [CA/CA]**; 600 University Avenue, Toronto, Ontario M5G 1X5 (CA).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **WRANA, Jeff** [CA/CA]; 106B Claremont Street, Toronto, Ontario M6J 2M5 (CA). **BARRIOS-RODILES, Miriam** [MX/CA]; 145 Marlee, Apartment 1212, Toronto, Ontario M6B 3H3 (CA).
- (74) Agent: **KURDYDYK, Linda**; McCarthy Tetrault, Suite 4700, P.O. Box 48, 66 Wellington Street West, Toronto Dominion Bank Tower (CA).
- (81) Designated States (national): **AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.**
- (84) Designated States (regional): **ARIPO** patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), **Eurasian** patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), **European** patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), **OAPI** patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:
— with international search report
- (88) Date of publication of the international search report: **15 July 2004**

[Continued on next page]

(54) Title: **METHODS FOR ASSAYING PROTEIN-PROTEIN INTERACTIONS**



(57) Abstract: The invention relates to methods and reagents for screening, identifying, and/or quantifying molecular interactions. In particular, the invention provides a method for identifying protein-protein interactions comprising prey proteins interacting with bait proteins comprising: (a) introducing one or more prey protein in cells, wherein a prey protein is labelled with an epitope tag permitting separation of the prey protein from other proteins in the cells; (b) introducing one or more bait protein in the cells, wherein a bait protein is labelled with a detectable substance permitting detection of protein-protein interactions comprising a prey protein and the bait protein; and (c) assaying for protein-protein interactions comprising a prey protein and bait protein by detecting the detectable substance.

BEST AVAILABLE COPY

WO 2004/023146 A3



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/03/01354

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N33/68 C12Q1/66

Rece

ST/PTO

04 MAR 2005

10/526733

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	YOSHIOKA K ET AL: "Agonist-promoted heteromeric oligomerization between adenosine A1 and P2Y1 receptors in living cells" FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 523, no. 1-3, 17 July 2002 (2002-07-17), pages 147-151, XP004371164 ISSN: 0014-5793 page 149, column 1, paragraph 4 page 149, column 2, paragraph 1 -page 150, column 2, paragraph 2 figures 2,3	1,2,7,8, 12,15, 16, 19-21, 23,24
Y	--- -/--	1-30



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

9 March 2004

Date of mailing of the international search report

08/04/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Weber, P

INTERNATIONAL SEARCH REPORT

International Application No

PCT/03/01354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KATO YOICHI ET AL: "A component of the ARC/mediator complex required for TGFbeta/Nodal signalling" NATURE (LONDON), vol. 418, no. 6898, 8 August 2002 (2002-08-08), pages 641-646, XP002272017 ISSN: 0028-0836 page 643, column 1, last paragraph -column 2, paragraph 2 page 645, column 2, paragraph 8 - last paragraph figure 4	1,12-16, 24,26-30
Y	----	1-30
X	SHI XINGMING ET AL: "Hoxa-9 represses transforming growth factor-beta-induced osteopontin gene transcription" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 276, no. 1, 5 January 2001 (2001-01-05), pages 850-855, XP002272018 ISSN: 0021-9258 page 851, column 2, paragraph 2 page 853, column 2, line 37 -page 854, column 1, line 10 figure 6	1,12, 15-17, 23,24, 27-30
Y	----	1-30
X	SMITH DARRIN P ET AL: "LIP1, a cytoplasmic protein functionally linked to the Peutz-Jeghers syndrome kinase LKB1" HUMAN MOLECULAR GENETICS, vol. 10, no. 25, 1 December 2001 (2001-12-01), pages 2869-2877, XP002272019 ISSN: 0964-6906 page 2871, column 2, last paragraph -page 2872, column 1, paragraph 3 page 2874, column 1, last paragraph -column 2, paragraph 1 page 2876, column 1, paragraph 4 figures 2,5	1,5,6, 15-17, 23,24, 26-30
Y	----- -/--	1-30

INTERNATIONAL SEARCH REPORT

International Application No

PCT/03/01354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ITO T ET AL: "A comprehensive two-hybrid analysis to explore the yeast protein interactome" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 98, no. 8, 10 April 2001 (2001-04-10), pages 4569-4574, XP002958850 ISSN: 0027-8424 the whole document	5,6,16, 17,22, 24,25
Y	---	5,6, 16-30
X	UETZ P ET AL: "A COMPREHENSIVE ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS IN SACCHAROMYCES CEREVISIAE" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 403, 10 February 2000 (2000-02-10), pages 623-627, XP000938868 ISSN: 0028-0836 the whole document	5,6,16, 17,22, 24,25
Y	---	5,6, 16-30
X	GAVIN A-C ET AL: "Functional organization of the yeast proteome by systematic analysis of protein complexes" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 415, January 2002 (2002-01), pages 141-147, XP002958851 ISSN: 0028-0836 the whole document	5,6,16, 17,22, 24,25
Y	---	5,6, 16-30
X	HO Y ET AL: "Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 415, 10 January 2002 (2002-01-10), pages 180-183, XP002250082 ISSN: 0028-0836 the whole document	5,6,16, 17,22-25
Y	---	5,6, 16-30
Y	WO 02 29417 A (ODYSSEY PHARMACEUTICALS INC) 11 April 2002 (2002-04-11) the whole document	1-30

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/03/01354

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LI Z-R ET AL: "Differential expression of proteins regulating cell cycle progression in growth vs. differentiation" BIOCHIMICA ET BIOPHYSICA ACTA. MOLECULAR CELL RESEARCH, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 1356, no. 2, 24 April 1997 (1997-04-24), pages 149-159, XP004277577 ISSN: 0167-4889 the whole document	1-30
Y	BIANCO CATERINA ET AL: "Cripto-1 activates nodal- and ALK4-dependent and -independent signaling pathways in mammary epithelial cells" MOLECULAR AND CELLULAR BIOLOGY, vol. 22, no. 8, April 2002 (2002-04), pages 2586-2597, XP002272527 ISSN: 0270-7306 page 2587, column 2, paragraph 4 page 2588, column 1, last paragraph page 2588, column 2, last paragraph -page 2589, column 1, paragraph 1 page 2589, column 2, last paragraph -page 2590, column 1, paragraph 1 page 2591, column 2, last paragraph -page 2592, column 2, last paragraph figures 2,4,8	1-30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 03/01354

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 31 (completely), 1-4, 7-30 (partially)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box I.2

Claims Nos.: 31 (completely), 1-4,7-30 (partially)

Present claims 1-4,7-30 relate to a compound defined by reference to a desirable characteristic or property, namely "being detectable".

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT for only one of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the detectable compound used in Example 1 and Example 2 of the present application and referred to in dependent claim 21: Renilla luciferase.

Present claim 31 relates to a compound defined by reference to a desirable characteristic or property, namely "being identifiable with a claimed method".

The claim covers all compounds having this characteristic or property, whereas the application provides no support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for such compounds. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/03/01354

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0229417 A	11-04-2002	AU 1144402 A	15-04-2002
		WO 0229417 A2	11-04-2002
		US 2002064769 A1	30-05-2002

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☒ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.